Laboratory Testing Plays an Important Role in the Determination of Local Transmission of Zika Virus in Florida

Stephen L. White[1], Darryl Pronty[1], Edhelene Rico[2], Danielle Fernandez[2], Isabel Griffin[2], Andrea Bingham[3], Danielle Stanek[3], Carina Blackmore[4], Anna Likos[5], Marie-Claire Rowlinson[6], and Leah D. Gillis[1]

[1]Bureau of Public Health Laboratories, Miami, FL, [2]Florida Department of Health in Miami-Dade County, Miami, FL, [3]Bureau of Epidemiology, Tallahassee, FL, [4]Division of Disease Control and Health Protection, Tallahassee, FL, [5]Florida Department of Health, Tallahassee, FL, [6]Bureau of Public Health Laboratories, Jacksonville, FL

asm microbe 2017
CPHM LB6
Saturday, June 3, 2017
12:15PM to 2:15PM

Abstract

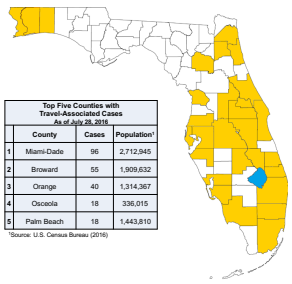
Background: Zika virus is an arbovirus that causes mild or asymptomatic infection in most humans. Like other arboviruses, symptomatic cases may experience fever, rash, and joint pain. Notably, ZIKV can be passed from a pregnant woman to her fetus causing birth defects, thus making the diagnosis and concomitant transmission analysis of infection crucial. As the Zika virus has spread through the Americas since 2015, U.S. public health laboratories have been prepared to perform testing of travel-related and potential locally acquired (non-travel related) cases.

Methods: In July 2016, the Florida Department of Health in Miami-Dade County investigated two cases of non-travel related Zika. It was determined that these were locally acquired cases from vector-borne transmission in the Miami-Dade area. This finding necessitated the active case finding of mild or subclinical cases through community surveying. Determination of unknown positive cases was performed by the CDC's Trioplex Real-time RT-PCR Assay, which is FDA-approved under an Emergency Use Authorization for the qualitative detection and differentiation of RNA from Zika, dengue, and chikungunya viruses.

Results: Because of the prolonged viremia displayed in urine and the ease of collection in the field setting, urine was determined to be the ideal specimen of choice to detect spread of the virus. Between July 26 and December 9, 2016, 1,885 urine specimens were collected as part of community "urosurveys." Of these, 29 cases of ZIKV infection were detected by PCR, each triggering an epidemiological investigation to ascertain the location of exposure.

Conclusion: Florida public health laboratories have been at the forefront of laboratory diagnostic testing for ZIKV. The testing of urine during community surveys with a rapid real-time PCR assay allows for the expedited identification of ZIKV cases and the swift public health response needed to ensure control of the spread of the virus.

Background

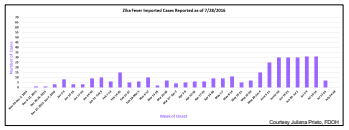


Top Five Counties with Travel-Associated Cases As of July 28, 2016

	County	Cases	Population[1]
1	Miami-Dade	96	2,712,945
2	Broward	55	1,909,632
3	Orange	40	1,314,367
4	Osceola	18	336,015
5	Palm Beach	18	1,443,810

[1]Source: U.S. Census Bureau (2016)

Governor Rick Scott directs the State Surgeon General to declare a public health emergency on February 9, 2016. By July 28, 2016, 29 of 67 counties in Florida had identified at least one case of Zika virus disease.



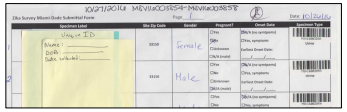
Within those 29 counties, 350 cases had been reported, 55 of which involved pregnant women. All cases investigated were determined to have been acquired abroad. On July 29, 2016, the Florida DOH announced that an investigation of a cluster of 4 cases indicated the disease was acquired locally.

Field Collection

Collection of urine specimens was coordinated by DOH-Miami-Dade and supported by the Bureau of Epidemiology. Deployed staff from other FDOH organizations, members of the county's Medical Reserve Corps, volunteers, and interns assisted with the collection of specimens.

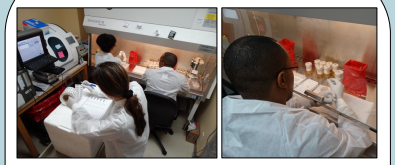
Two main models of specimen collection were employed: door-to-door sampling and sampling from a fixed location in the community. Door-to-door specimen required a considerable amount of staff and logistical support, whereas fixed location collections were less labor-intensive.

Specimens were kept refrigerated or on gel ice packs in coolers following collection. Couriers were used to transport specimens from the field to the laboratory.



Forms were optimized to ensure accurate data collection and facilitate accessioning in the laboratory. A dedicated refrigerator was setup in the laboratory specifically for urosurvey specimen drop-off and to keep ice packs frozen for the following day.

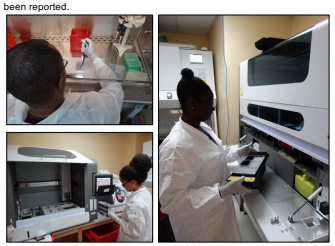
Accessioning & Extraction



Specimens were typically accessioned the following day. Whenever possible, two technicians and one support staff member accessioned specimens:

- The first technician verified specimen name, DOB, and specimen ID number
- The second technician prepared aliquots
- The support staff member assigned laboratory IDs

Two aliquots of each specimen were prepared from each primary collection container. This allowed for the manipulation of many specimens in a confined space at one time and simplified the archival process. Original specimens were not discarded until final results had been reported.



All specimens were manipulated in a BSC and lysed externally (before extraction) as an added safety measure. Two automated extraction methods were used:

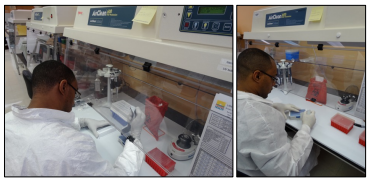
- Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit
- Rohce MagNA Pure LC Total Nucleic Acid Isolation Kit

The MagNA Pure LC 2.0 can extract approximately 32 specimens every 1.5 hours whereas the MP 96 System can extract approximately 96 specimens every hour.

Real-Time RT-PCR

The CDC's Trioplex Real-time RT-PCR Assay was used to detect RNA from ZIKV in specimens. Testing of urine specimens submitted without a paired serum contradicted the FDA's EUA and was performed under a public health exemption for surveillance. The assay utilizes dual-labeled hydrolysis probes (TaqMan) for the detection and differentiation of dengue, chikungunya, and Zika viruses in clinical specimens. Two reactions are used for each specimen: one multiplexed for the detection of the three viruses and one for the detection of RNase P.

Marker	Dye	Quencher	Target
Dengue	FAM	BHQ-1	5'-UTR
Chikungunya	HEX	BHQ-1	nSP1
Zika	Texas Red	BHQ-2	Envelope Gene
RNase P	FAM	BHQ-1	Human Ribonuclease P gene



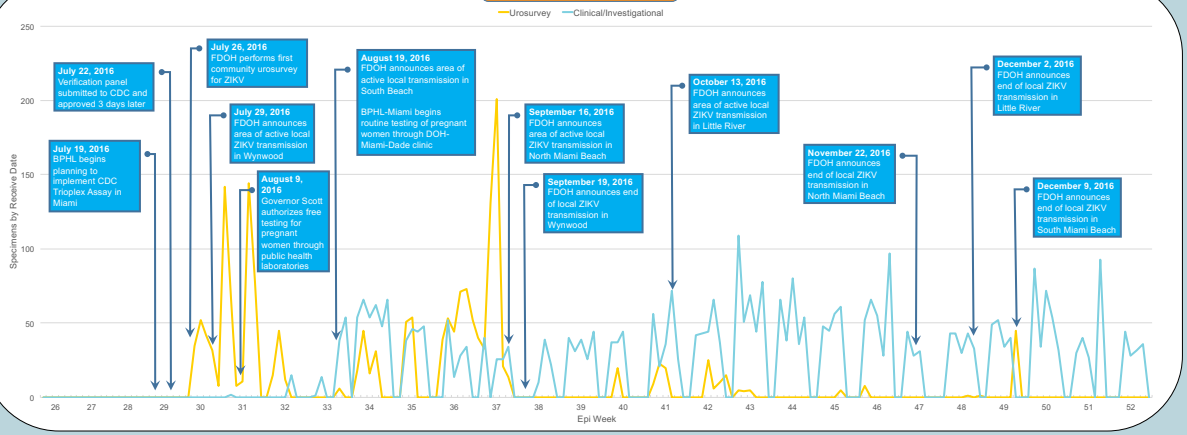
Several strategies were employed to maximize capacity while minimizing errors:

- Standardized PCR worksheets were used to ensure no miscalculations were made with master mix recipes
- Master mix was pipetted using a multichannel pipette to limit mistakes when making multiple plates
- Specimen extracts were also pipetted with a multichannel pipet to minimize confusion and technician fatigue when setting up multiple plates

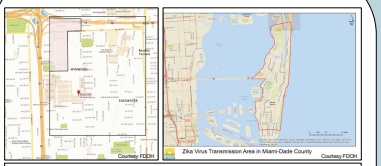
Purpose	Detected	Not Detected	Inconclusive	Rejected	Total
Urosurvey	29	1851	1	4	1885
Investigation	39	350	0	0	389
Clinical	1	3380	0	0	3381
Total	69	5581	1	4	5655

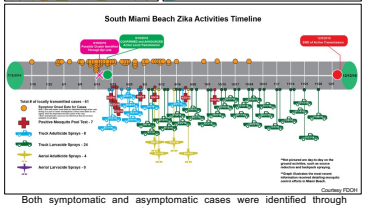
In addition to testing urosurvey specimens, the laboratory began routine testing of pregnant women in the county and continued supporting epidemiological investigations.

Specimens Tested
July-December 2016



Public Health Actions

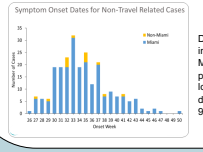




Both symptomatic and asymptomatic cases were identified through community urosurveys. Information from these urosurveys, along with other surveillance activities, guided public health actions such as:

- Identify clusters
- Informing mosquito control activities
- Identifying areas and timelines of active transmission
- Advising at-risk populations of the risks associated with travel to certain areas
- Performing environmental assessments for mosquito activity

The information gained through community urosurveys- both positive results as well as negative- helped provide situational awareness to decision makers, the media, and the community.



Distribution of Zika virus infection cases acquired in Miami-Dade County. Due, in part to these urosurveys, local transmission was declared over on December 9, 2016.

Conclusion

Conducting urosurveys presents many challenges beyond the normal collection and testing of specimens. The undertaking requires a large degree of coordination between both the laboratorians and epidemiologists to ensure the timely submission and testing of specimens.

Challenges for the field epidemiologist:

- Logistical challenges of organizing and managing urosurveys
- Implementing the appropriate collection strategy
  - Door-to-door sampling may offer a more systematic approach
  - Fixed sampling requires fewer staff members, but relies on the community
- Rapidly incorporating near real-time data to guide further investigations

Challenges for the laboratorian:

- Having the ability to handle a surge of specimens without excessive disruption to normal operations
- Maintaining an adequately trained staff
- Utilizing the proper instrumentation to adapt to the workload

The success of these urosurveys was a result of a collaborative effort between laboratorians and epidemiologists alike. Execution of urosurveys was pivotal to the public health actions taken and, ultimately, the control of the ZIKV in Miami-Dade County.

PHAB Accredited Health Department
Public Health Accreditation Board